Plant immunisation: from myth to SAR†

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Abstract: The idea that plants might be able to develop a form of acquired immunity to infection following exposure to a pathogen has been current ever since discovery of the animal immune system in the later years of the nineteenth century. Early attempts to demonstrate a comparable system in plants focused on the detection of precipitating antibodies and hence were doomed to failure. Nevertheless, largely anecdotal evidence for plant immunisation continued to accumulate, culminating in the discovery of phytoalexins in the 1940s. Convincing evidence for systemic changes in plant resistance following an inducer inoculation was not available until 20 years later, when pioneering work on tobacco infected with blue mould (*Peronospora tabacina*) or tobacco mosaic virus (TMV) showed that tissues remote from the inoculation site were altered in disease reaction type. Increased resistance was expressed as a reduction in lesion numbers and size, and a reduced rate of pathogen reproduction. Systemic acquired resistance (SAR) has now been demonstrated in at least 20 plant species in at least six plant families, although detailed genetic or molecular analysis has mainly been confined to a few models, such as tobacco, cucumber and *Arabidopsis*. SAR is associated with the coordinate induction of genes encoding defence proteins which can be used as molecular markers of the response. The availability of *Arabidopsis* mutants altered in the induction and expression of SAR is now providing new insights into the signal transduction pathway(s) involved, and will enable comparison with the molecular mechanisms operating in other plant taxa. Important unresolved questions concern the nature of the translocated signal, the mechanism of defence 'priming', efficacy of the response against different pathogens, and practical exploitation of SAR in crop protection. The first generation of chemical plant defence activators is now commercially available and optimal use of these SAR inducers in integrated disease control requires further evaluation. The prospects for engineering transgenic crops altered in the regulation or expression of SAR is also a subject for further investigation.

Keywords: induced resistance; phytoalexins; PR proteins; defence signalling

1 INTRODUCTION

The notion that plants might be able to develop a form of acquired immunity to infection following exposure to a pathogen, or antigenic substances derived from a pathogen, has existed ever since discovery of the animal immune system in the later years of the nineteenth century. The early history of research on plant immunity has been comprehensively reviewed by Chester.[1] Most experiments sought to demonstrate the presence of precipitating antibodies in agglutination tests, and proved inconclusive due to the widespread occurrence in plant extracts of cross-reactive proteins, such as lectins, which gave non-specific binding reactions. Nevertheless evidence was obtained that plant 'vaccination' could lead to changes in the response of tissues to a subsequent microbial challenge. Attempts to show that this phenomenon was due to the production of specific antibodies equivalent to those found in animals were, with the benefit of hindsight, doomed to failure.

One conclusion arising from this early work on plant immunity is that enhanced resistance to the pathogen is normally confined to the site of initial inoculation, and is localized rather than systemic, unlike the animal response. A mechanistic basis for this observation was provided by the discovery that plant tissues subjected to stress or pathogen attack accumulate low-molecular-weight antimicrobial compounds, termed phytoalexins. The induction and regulation of phytoalexin biosynthesis has been extensively analysed,[2] although it is now clear that phytoalexins are only one component of a complex cascade of defence responses responsible for localizing infection.

† Based on a paper presented at the meeting 'Systemic Acquired Resistance', organised by JM Smith and AH Cobb on behalf of the Pesticides Group of the SCI and held on 10 March 1998 at 14/15 Belgrave Square, London.

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Contract/grant sponsor: Biotechnology and Biological Sciences Research Council

(*Received 10 March 1998; revised version received 10 August 1998; accepted 30 September 1998*)

Table 1. Some biological characteristics of systemic acquired resistance

1. Induced by agents or pathogens causing necrosis e.g. local lesions.
2. Delay of several days between induction and full expression.
3. Protection conferred on tissues not exposed to inducer inoculation.
4. Expressed as reduction in lesion number, size, spore production, pathogen multiplication etc.
5. Protection is long-lasting, often for weeks or even months.
6. Protection is non-specific ie effective against pathogens unrelated to inducing agent.
7. The signal for SAR is translocated and graft-transmissible.
8. Protection not passed on to seed progeny; transmission to vegetatively propagated tissues has not been fully resolved.

2 EXPERIMENTAL VALIDATION OF SYSTEMIC ACQUIRED RESISTANCE (SAR)

The first convincing evidence that plants can also develop systemic resistance to pathogens was obtained in experiments with tobacco. The downy mildew pathogen *Peronospora tabacina* Adam normally infects leaves, causing blue mould disease. Inoculation of stem tissues induces resistance to blue mould in leaves above the inoculation site.[3] Development of a localized necrotic lesion due to growth of the pathogen in the outer stem tissues appears to be important for the induction of resistance, as injection of heat-killed or sonicated inoculum is ineffective.[4] Protection develops over a three-week period following inoculation, and is highly effective, reducing infection by over 90% compared with unprotected control plants. This inoculation has also been shown to control disease under field conditions, comparing favourably with treatment with the best available fungicide, metalaxyl.[5] In this system, protection is long-lasting, essentially for the remainder of the growth period of the plant. The increased resistance is not, however, passed on through seed. The persistence of SAR is clonally propagated tissues of tobacco has not been fully resolved; Lucas *et al*[6] found that regenerants propagated from shoot tips or leaves of protected plants were as susceptible to blue mould as equivalent plants from non-induced controls, while Tuzun and Kuc[7] reported significant differences in the resistance of regenerants cultured from young leaves of protected versus non-induced plants. One possible explanation for these contrasting results is that the inoculum concentrations and inoculation methods used as a challenge in the two studies were different.

Ross[8] showed that inoculation of one leaf of a tobacco cultivar possessing local lesion resistance to TMV with the virus increased the resistance of other leaves on the same plant to TMV. The altered response was expressed as a reduction in the number and size of local lesions formed. Later work showed that the inducer inoculation not only boosted resistance to TMV, but also to unrelated fungal and bacterial pathogens. For instance, McIntyre *et al*[9] reported results of experiments in which inoculation of a hypersensitive (ie local lesion) tobacco cultivar with TMV induced resistance to two oomycete fungi, *Phytophthora parasitica* Dastur and *Peronospora tabacina*, and the wildfire bacterium *Pseudomonas tabaci* Stevens, as well as to TMV. The reproduction of the aphid *Myzus persicae* Suly. was also reduced on TMV-inoculated plants. This established one of the most important differences between plant SAR and animal immunity, that is, the apparent lack of specificity of the plant response.

In a series of papers, Kuc and colleagues showed that SAR can also be induced in bean[10] and cucumber.[11,12] In the latter host the biological spectrum of protection conferred by SAR includes pathogens as diverse as downy and powdery mildews, vascular wilt fungi, foliar anthracnose (*Colletotrichum lagenarium* (Paserini) Ell. & Holst.), bacterial leaf spot and wilt, tobacco necrosis virus (TNV) and cucumber mosaic virus (CMV). Table 1 summarizes the main biological features of SAR, as demonstrated in these early studies.

To date, SAR has been shown in at least 20 plant species from at least six different plant families.[13] Some type of systemic resistance has also been demonstrated with mites and insects.[14,15] Important questions remain, however, about the extent to which the mechanisms responsible for the induction and expression of SAR are conserved between different plant taxa. This question can only be resolved by identifying the different pathways and genes involved in the response.

3 MECHANISTIC ANALYSIS OF SAR

Once SAR had been shown to be a reproducible fact of life in the plant kingdom, attention shifted to the mechanisms underlying this form of resistance. A simple model of SAR envisaged that the initial inducer treatment generates a signal in the exposed tissues, which is then translocated to remote parts of the plant, where cells are somehow primed to resist the second, challenge inoculation. Key questions concern how the inducer treatment is transduced into a signal, the identity of the signal molecule(s) itself, and the mechanism of enhanced resistance in the primed tissues. Recent progress towards answering these questions in detail will be covered elsewhere, so this review will be confined to a few general points. Important technical advances for the analysis of SAR were (1) the demonstration that systemic resistance occurs in *Arabidopsis*,[16] providing the opportunity for a molecular genetic approach to the problem,[17] and (2) the use of transgenic plants

Table 2. Some landmarks in SAR research

		Reference
1933	Chester review of plant immunity	1
1959	Field observations on tobacco SAR	3
1960	SAR to tobacco blue mould	3
1961	SAR to tobaccco mosaic virus	8
1975	SAR in Cucurbitaceae	11, 12
1982	SAR in barley	30
1989	Systemic resistance to insect attack	14, 15
1992	SAR in Arabidopsis	16
1994	A central role for salicylic acid	18
1995	First commercial SAR activator	23

expressing salicylate hydroxylase to establish the key role of salicyclic acid (SA) in plant defence.[18] The development of systemic resistance is now known to be associated with coordinate expression of a set of genes encoding defence-related proteins, and these serve as useful molecular markers of the response.[19] However, complex issues remain concerning the relationship between SAR and plant cell death during induction of the response, the potential role of signal molecules other than SA, such as systemin, ethylene and jasmonates, and why some pathogens are apparently unaffected by SAR.[20]

Table 2 summarizes some of the landmarks in SAR research which have established the phenomenon as an important aspect of active plant defence against pests and pathogens.

4 PRACTICAL EXPLOITATION OF SAR

The agrochemical industry has a long-standing interest in discovering compounds which might act indirectly against pathogens via endogenous plant defence pathways. Indeed, some compounds initially described as fungicides are now known to potentiate defence responses rather than having a direct effect on the pathogen, for example probenazole.[21] Biochemical and molecular elucidation of SAR has provided the opportunity for a more targeted approach to the discovery of plant defence activators. Novel screening techniques identified the benzothiadiazoles as a new class of chemicals which activate SAR,[22] and one derivative (CGA 245704, Bion®) has now gone through to commercial launch.[23]

The novel mode of action of plant defence activators suggests that they will be of great potential value in the integrated control of plant diseases, especially where resistance to conventional fungicides is a problem. As these compounds act on the endogenous defence pathways of the plant, they provide broad-spectrum disease control which should be durable. There are many unresolved questions about their use, however, such as optimal timing and method of application on different crops, integration with other types of pesticide, and interactions with the physiology of the plant. These chemicals are, by definition, altering plant gene expression and metabolism, with possible effects on crop growth and development. There also appear to be intriguing differences in the efficacy of chemical defence activators on different crop species. Whether such differences are due to variations in uptake or dose response between species, or reflect real differences in the induction and regulation of plant defence pathways, is not, as far as I am aware, currently understood. Further research also needs to be carried out to optimize the formulation and application of these chemicals on different types of crops. For instance, a recent small-scale study in my own laboratory has demonstrated the feasibility of using seed dressings with a plant defence activator to protect against seedling diseases;[24] the practicalities of this approach require further investigation in a commercial crop.

The other area of potential exploitation of SAR is through manipulation of the response in transgenic crops. Individual defence proteins induced during SAR, such as some PR proteins, have already been constitutively expressed in transgenic plants with some improvements in resistance to fungal infection.[25] A more interesting possibility is to alter the induction or regulation of the SAR pathway, so that plant tissues are already primed against attack. Several classes of mutants which constitutively express SAR have been identified in *Arabidopsis*,[17,26] showing that this phenotype can occur naturally. What is not known, however, is the effect of constitutive expression of SAR in a crop species in the field.

Irrespective of the immediate practicalities of exploiting SAR in crop protection, a better understanding of the phenomenon, and especially its relationship to other active plant-defence response, will be of value in developing genetic strategies for boosting crop resistance to pathogens and pests.

5 SAR IN NATURAL PLANT COMMUNITIES

Little is known about the extent and significance of SAR in natural ecosystems as opposed to crops.[27] As far as I am aware no-one has sampled wild populations of plants exposed to pathogen or pest attack for the occurrence of SAR-related gene expression. Wild plants presumably have the potential to develop SAR, but it is not clear if inducing conditions occur regularly in natural environments. Non-pathogenic organisms, such as rhizosphere bacteria and mycorrhizas, can elicit plant defence, so perhaps there is some background level of induced resistance expressed in wild plant communities. The recent demonstration that volatile salicylate analogues can provide an airborne signalling mechanism activating disease resistance in plants adjacent to those undergoing a necrotic reaction to virus infection raises the intriguing possibility that groups of plants can respond collectively to a perceived biological threat.[28] Plants in natural environments are exposed to a wide variety of biotic agents, and there may be

subtle differences in the induction and expression of resistance between the different types. This has already been shown in experiments on induced resistance to different classes of herbivorous arthropods.[29]

ACKNOWLEDGEMENTS

I thank John Hargreaves for constructive comments on the manuscript. IACR-Long Ashton Research Station receives grant-aided support from the Biotechnology and Biological Sciences Research Council of the UK.

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